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OBSERVATIONS UPON FLAGELLATED MALARIAL PLASMODIA.

DESCRIPTION OF TWO VARIETIES.

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Ever since the discovery of the plasmodium of malaria by Laveran the nature and significance of the flagellated organism have constituted a mooted question. Held by some authorities to be of a degenerative nature, and by others as evidences of vital activity, the flagella have been closely studied, and a considerable literature has been accumulated concerning them. In the light of recent research, it can no longer be doubted that the evolution of the flagellated organism is a vital and most important phase in the life history of the plasmodium, and that the theory that it is a degenerative body must be abandoned.

During the past seven months I have had the opportunity of studying the blood of a large number of sol-

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diers returning from Cuba and suffering from tertian and æstivo-autumnal malarial fevers, and one of the points of greatest interest has been the study of flagellated organisms. As the result of these studies I believe that there occur in the blood of cases of tertian, and especially in the blood of cases of æstivo-autumnal fever, two forms, or perhaps varieties, of the flagellated organism. Before describing them, a few words regarding the best way of obtaining flagellated organisms may be of interest.

Method of Obtaining Flagellated Organisms.—It is a well-known fact that under ordinary circumstances flagellated plasmodia do not appear in a specimen of blood until some time after it has been removed from the body, generally from fifteen to twenty minutes. This rule has exceptions, however, for I have seen flagellated organisms in a blood specimen which had only been removed two minutes from the circulation; this is a rare exception, and even in specimens long removed from the body flagellated bodies are not always found. I have found the following method of obtaining these bodies at once simple and effective. The finger or ear is carefully cleansed with alcohol, as are also the slides and cover glasses. A small elastic band is now placed around the finger, or, if the lobe of the ear is used, it is compressed by an assistant. The puncture is made with a sterile needle or lancet and the first drop of blood wiped away. A second drop is now squeezed out and allowed to remain exposed to the air until the slide is breathed gently upon by the operator, when the tip of the drop of blood is gently pressed upon the surface of the slide which has been breathed upon. The cover glass is then immediately placed over it, and the prepa-

ration is ready to examine. The slight exposure to the air, and the small amount of moisture upon the slide caused by breathing upon it, seem to hasten exflagellation, for specimens so prepared almost invariably contain flagellated bodies. In specimens prepared in this way I have seen as many as twenty flagellated bodies in one preparation, and have often observed two in a single field. In my observations upon the bodies in question I have used this method exclusively, and have never experienced any difficulty whatever in finding material —*i. e.*, flagellated bodies—for study.

Description of Flagellated Forms.—As has been stated in the early part of this paper, in many cases of tertian malarial fever, and almost invariably in cases of æstivo-autumnal fever, I have observed two seemingly distinct forms of the flagellated bodies. By this statement it is not meant that every case of tertian or æstivo-autumnal fever examined showed these forms, but that the cases showing flagellated bodies presented them. While the two forms were often noted in tertian fever, they were by far the most common in æstivo-autumnal fever, and, as flagellation is generally conceded to be of vital importance in the life history of the parasite, this fact agrees with the well-known tenacity of the æstivo-autumnal infection. These two forms of flagellated parasites I have ventured to designate respectively as the active and passive flagellated plasmodium.

Description of the Active Flagellated Plasmodium (Tertian Fever).—In examining a specimen of blood from tertian fever a number, sometimes only one or two, of large, almost circular, bodies are noted, with the characteristic pigment distributed over the surface, and in

very active motion. The motion is aptly described as dancing, and is very much more rapid than that ordinarily present in the parasite, and at once attracts attention for that reason. Quite often, however, these swollen bodies will be seen, the pigment when first noticed being immotile, but suddenly developing very active motility while being observed. When the pigment is immotile it seems to be collected in small blotches or spots within the protoplasm of the parasite, but when in motion it is distributed quite evenly throughout the protoplasm (see Fig. 1, *A* and *B*). Besides the very active motion of the pigment granules, if the edge of the parasite is carefully watched, it will be seen that it also is in motion, undulating and protruding, as though, as Richard said, something contained within the parasite were trying to escape. In a variable length of time—from five minutes to half an hour or more—the

FIG. 1.—*A*. Large swollen pigmented body showing pigment in blocks. *B*. Large swollen pigmented body showing pigment evenly distributed.

pigment will be seen to collect more centrally, the motility being somewhat lessened, and instantly, like an explosion, there appear at certain portions of the edge of the parasite long, thin, colorless, actively moving filaments, which undulate rapidly, lashing about among the red corpuscles, to which they impart sometimes a peculiar spinning motion. The filaments may number from one to five, and are usually long and thin, having a

slightly clubbed extremity (see Fig. 2). The junction of the flagellum with the parasite is not visible, it seeming to be continuous with the periphery of the organism.

Besides the clubbed extremity, the flagella some-

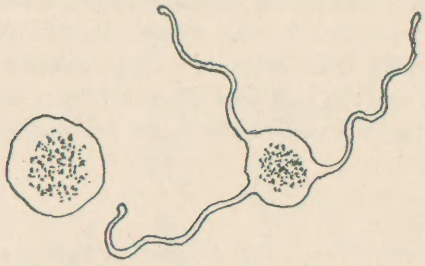

FIG. 2.—*A*. Parasite just prior to flagellation. *B*. Flagellated parasite showing clubbed ends of flagella.

times show small nodular swellings in their course, and also a few grains of pigment, which may be distributed along them or collected at the extremity (Fig. 3). They generally measure about two or three times the diameter of the parasite from which they spring, but sometimes may be even longer. There also occur shorter, stouter forms, having a more sluggish, serpentine motion (Fig. 3).

There may now occur one of several things: either the flagella may break loose from the parent parasite, or they may become gradually motionless and disappear, or they may fold themselves around the parasite, which shrinks and degenerates, or the parasite may itself fragment and the flagella degenerate and disappear.

In the first case, after the flagellum has lashed about among the blood-corpuscles for a variable length of

time, seemingly trying to free itself from the mother parasite, it at last succeeds, and swims off in a serpentine manner among the red corpuscles (Fig. 4). In some cases the flagellum tugs very vigorously in its efforts to free itself, actually pulling the mother parasite about, but, of course, for only a minute distance. After it has become free it may exhibit motion for a long time, even an hour or more. If it chances that the flagellum was the only one given off from the mother parasite, the pigment of the latter becomes motionless,

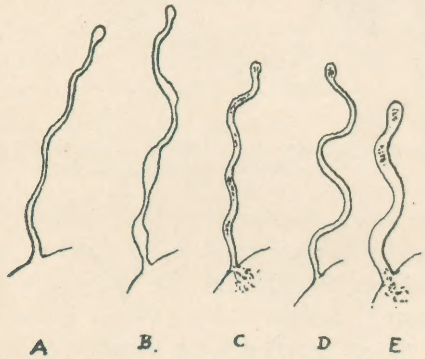

FIG. 3.—Types of flagella. *A*. Flagellum showing clubbed extremity. *B*. Flagellum showing nodular swellings. *C*. Flagellum showing pigment within it and at its extremity. *D*. Flagellum showing pigmented extremity. *E*. Flagellum short, thick, and pigmented.

the parasite quickly shrinks, and soon only a mass of pigment and degenerated material remains (Fig. 4).

In the second case, after persisting for some time, from half an hour to an hour or more, the flagella gradually become motionless and disappear, while the pigment ceases moving and the body of the parasite shrinks, becomes vacuolated, and soon presents a mere clump of *débris*.

In the third case, after a certain time, the flagella become less actively motile and seem to become entangled with the body of the parasite, thus seeming to be folded about it, sometimes loops being formed by the attachment of a flagellum to the mother parasite. The pigment in the body of the parasite becomes motionless

FIG. 4.—*A*. Free flagellum which has just escaped from parasite. *B*. Shrunken degenerated parasite after exflagellation.

FIG. 5.—*A*. Tertian parasite with flagellum folded upon it. *B*. Tertian parasite with flagella forming loops about it.

and the same degenerative changes occur as in the former case (Fig. 5).

In the last case the parent parasite, either after the escape of one or more flagella or in cases where no escape takes place, breaks up into two or more parts, each part containing pigment, which remains in active motion for some time. On careful observation it will be seen that the fragments are united by very delicate hyaline threads. The flagella soon lose their motility and disappear, but the fragments containing the pigments may persist for a long while. In one case the pigment was seen to be in active motion in one of the fragments of such a parasite after a period of eight hours, at a room temperature of 70° F.

In Fig. 6 is sketched the process of fragmentation as observed in a flagellated organism.

The form of flagellated organism just described is the most common form found in the blood of tertian malarial fever. The same form is also found in æstivo-autumnal fever, but varies somewhat in morphology, but in unessential particulars.

In æstivo-autumnal malarial fever the active flagellated form of the malarial parasite is smaller in size than is the same form in tertian malaria, is more oval

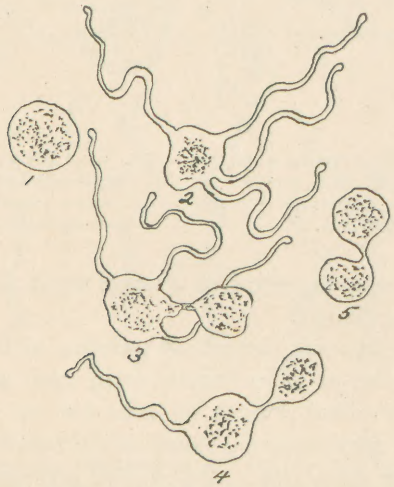

FIG. 6.—Fragmentation as observed in tertian flagellated parasites. *1*. Parasite prior to flagellation. *2*. Parasite showing four flagella. *3*. Parasite becoming fragmented into two portions. *4*. Further development of fragmentation, all but one of the flagella having disappeared. *5*. Fragmentation complete. All the flagella have disappeared, and the parasite has divided into two nearly equal portions.

in outline, as a rule, and the pigment is somewhat more coarse, and sometimes arranged in a wreathlike form. These differences are illustrated in Fig. 7. The same phenomena are observed here as in the case of the ter-

tian flagellated organism, and which have just been described.

The chief facts to be noticed in our study of the form of flagellated malarial parasite which we have chosen to call the active form are: the extreme activity of the pigment in the parasite prior to exflagellation and during the time the flagella remain attached to

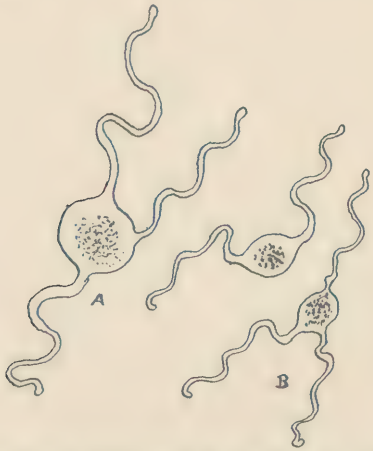

FIG. 7.—*A*. Flagellated tertian malarial parasite. *B*. Flagellated æstivo-autumnal malarial parasite, showing smaller size, more oval outline, and more compactly collected pigment.

it; the clubbed extremity of the flagella, and the separation and consequent individual existence of the flagella, and their power of individual and progressive movement in the blood.

Description of the Passive Flagellated Plasmodium (Æstivo-autumnal Fever).—The form about to be described occurs also in tertian malarial fever, but in much smaller numbers than in æstivo-autumnal fever, so that I have chosen to describe it as it occurs in the latter

form of fever, the only difference being that it is larger in the tertian fever. Before describing it, however, it should be understood that not every case of æstivo-autumnal fever shows flagellated organisms. They will only be found in the peripheral blood in those cases of fever showing crescents, for it is from the crescent that they develop. It is not often that one has the good fortune to witness the development of the crescent into a flagellated organism, and the following observation is therefore of importance. In observing a sample of blood from a case of æstivo-autumnal fever which was particularly rich in crescents, I observed a medium-sized crescent entirely inclosed within a red corpuscle, occupying about one half of it. While under observation the crescent suddenly seemed to expand into a round body, the pigment, motionless, occupying the centre. After about ten minutes this round body suddenly became free, the red corpuscle seeming to melt away from around it, remaining as a pale shell close to the now free round body. Within a short time, probably about five minutes, the pigment within the round body became motile, at first slowly, then very rapidly, distributed throughout the protoplasm of the organism, and suddenly two flagella shot forth from opposite sides of the parasite. One of these afterward became detached and was lost sight of; the other, after persisting for about half an hour, ceased moving, and it and the body of the parasite became shrunken and degenerated. This observation conclusively proves two things: First, that the crescents are developed within the red blood-corpuscles, and second, that the active flagellated parasite is developed from the crescents (see Fig. 8). It will be noticed that in the above observation it was

the active flagellated parasite which was developed, and not the passive form, which will now be described.

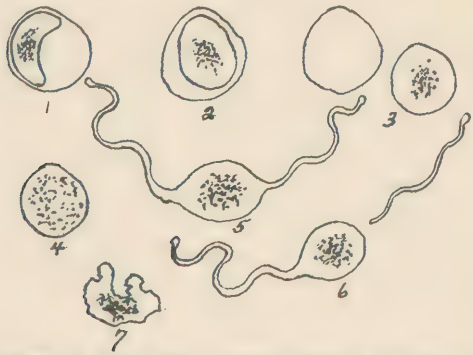

FIG. 8.—Process of flagellation in æstivo-autumnal parasite. *1.* Crescent inclosed entirely within red blood-corpuscle. *2.* Crescent cell, having expanded into a round body within the red corpuscle. *3.* Round body, which has escaped from the red corpuscle which remains as a pale shell beside it. Pigment motionless. *4.* Round body with active pigment. *5.* Round body sending out two flagella. *6.* Round body with one flagellum detached. *7.* Shrunken and degenerated parasite.

The Passive Flagellated Parasite (*Æstivo-autumnal*).—I have chosen to describe this form as it occurs in æstivo-autumnal fever, because it is most numerous and most characteristic in the blood of such cases.

In the blood of those cases of æstivo-autumnal fever containing crescents will be noticed numerous round and oval bodies in which the pigment is arranged in a wreath-like form. These bodies seem to be of two kinds. First and most numerous are those in which the pigment is of rather dotlike or rodlike form, and fine, sometimes motile and sometimes not, and those, fewer in number, and almost always round, in which the pigment is arranged in large black or dark-brown dots, in a perfect circle, and almost never motile. From the first variety

arise quite often the active flagellated organism, which has already been described; from the last, the passive flagellated organism. These two forms are illustrated in Fig. 9.

The last-named round bodies merit a more minute description. They are clear cut and definite, perfectly circular, and appear to possess a somewhat granular protoplasm; the pigment is arranged in round, very dark dots, forming a perfect circle, which may, however, be arranged to one or the other side of the protoplasm of the organism. The pigment is generally non-motile, but occasionally a peculiar trembling motion

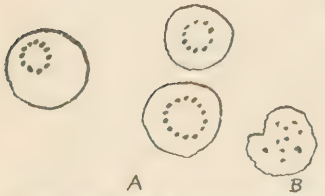

FIG. 9.—*A*. Round body with pigment in perfect ring. Seldom motile. *B*. Round body with pigment more evenly distributed and often motile.

FIG. 10.—*A*. Round bodies with pigment in wreath form. *B*. Oval bodies with pigment in wreath form. *C*. Round bodies with pigment in larger blocks.

may be observed; but the pigment retains its circular arrangement always, unless degenerative changes occur (see Fig. 10).

No matter how long such a round form is watched, it will never be seen to present the phenomena of exflagellation. The pigment does not become active and distributed throughout the protoplasm, nor do flagella emerge from within it. But in examining blood con-

taining these bodies some of them are seen to possess one, two, or more flagella. These flagella are somewhat peculiar. They seldom possess a clubbed outer extremity, as in the case of the active flagellated body, but at their juncture with the round body a nodule is often noted which resembles exactly the clubbed extremity of the flagellum of the active flagellated body. The movements, also, of these flagella are peculiar. Instead of the rapid serpentine lashings seen in the flagella as put forth from the active parasite, the movements were of a different character. The flagella seem to straighten and then relax, revolving apparently very rapidly upon their axes; sometimes they may be seen to pull themselves loose from the round body and again become attached to it. In the meanwhile the pigment within the round parasite has maintained its circular form, and is, at most, very slowly motile (see Fig. 11).

Sometimes no nodular swelling is noted at the point of attachment of the flagella, and in such parasites the pigment has a more rapid vibratory motion.

This same form occurs in tertian fever, but is larger, the pigment is less regularly arranged, and it is very much more rare.

The question arises, What do these forms of the malarial parasite signify? A few months ago, in a conversation with Dr. Thayer, he mentioned the occurrence of these peculiar flagellated bodies, and thus called my attention forcibly to the fact of their occurrence, which I had noted but had not paid much attention to. Since that time I have convinced myself that these forms occur, not as a rarity, but very often, and that they represent, as has been suggested by McCallum, the efforts of flagella, which have been set free in the

circulation, to penetrate into the interior of a parasite, represented by the round bodies just described. While I have not been so fortunate as have McCallum, Thayer, and others as to have actually seen the disappearance

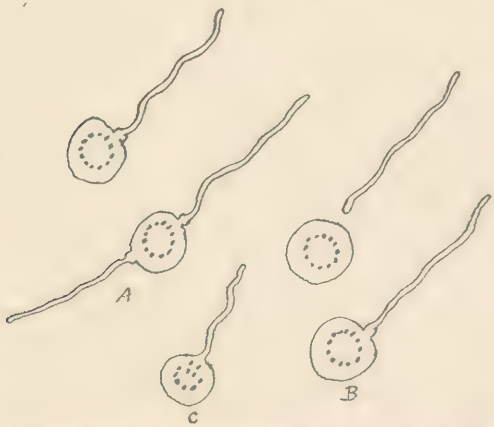

FIG. 11.—Passive flagellation as observed in æstivo-autumnal malarial parasite. In these figures note circular arrangement of the pigment, the flagella without clubbed ends, and straight outlines. In one figure a flagellum is seen just before attachment to the round body.

of a flagellum within one of these parasites, I am convinced that this does occur from what I consider sufficient evidence to prove the fact. From the appearance and character of the motion of the flagella, and the passive condition of the organism to which they are attached, it seems to be impossible to believe other than that these flagella are striving to push their way into the parasite, and the fact that flagella have been seen to become detached from the parasite and again attach themselves to it, is almost conclusive proof that such is the fact.

The circle of events is, then, I believe, as follows:

The *active flagellated organism* is developed from the full-grown tertian organism and the crescentic æstivo-autumnal organism. Flagella are produced and liberated, the mother organism, her duty fulfilled, degenerating and perishing. The free flagella swim actively about among the blood-corpuscles until they come in contact with the peculiar round, passive parasite, which they endeavor to penetrate. McCallum and Thayer have seen this penetration occur, one of the flagella becoming submerged, so to speak, within the substance of the parasite.

The nature of the process we are as yet ignorant of, but these two varieties of flagellated parasites do occur in the blood in malarial fever, and the observations upon them so far conclusively prove that the flagellate body is not a degenerative body, but is, without doubt, a very highly developed vital form of the plasmodium of malaria. It is but reasonable to suppose that these forms are calculated to preserve the life of the parasite outside of the human economy, as they arise only when the blood has been exposed to external conditions, and that we have in this process another proof of the extra-corporeal existence, in another form, of the malarial parasite.

My thanks are due Dr. Thayer for his suggestions regarding the forms described, and which prompted this research.

NOTE.—Since the writing of this article I have had the opportunity of confirming the observations noted at the United States General Hospital, Presidio of San Francisco, in cases of malarial fever in soldiers returning from the Philippine Islands. To Colonel A. C. Girard, commanding officer of that hospital, and to Major Charles Richard, late commanding officer of the Simpson General Hospital, my thanks are due for their kindly interest shown and the facilities offered for the study of the subject.